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(54) Title: IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES

(57) Abstract: Compounds useful as probes for determining the relative number and/or function of specific receptor subtypes. Of particular interest are nicotinic agonists and antagonists (e.g., metanicotine-type compounds and azaadamantane-type compounds) that are selective to certain nicotinic receptor subtypes. Those compounds are labeled with a radioactive isotopic moiety such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I or ^{125}I. Central nervous system disorders are diagnosed by administering to a patient a detectably labeled compound, and detecting the binding of that compound to selected nicotinic receptor subtypes (e.g., alpha 7 and/or alpha 4 beta 2 receptor subtypes). The compounds that have been administered are detected using methods such as position emission topography (PET) and single-photon emission computed tomography (SPECT). The present invention is useful in the diagnosis of a wide variety of CNS diseases and disorders, including Alzheimer's disease, Parkinson's disease and schizophrenia.

IMAGING OF NICOTINIC ACETYLCHOLINE RECEPTOR SUBTYPES

BACKGROUND OF THE INVENTION

The present invention relates to diagnostic compositions, and particularly to those compositions incorporating compounds that are capable of affecting certain selected nicotinic cholinergic receptor subtypes. The present invention also relates to probes for diagnosing a wide variety of conditions and disorders, including conditions and disorders associated with dysfunction of the central and autonomic nervous systems.

Nicotine has been proposed to have a number of pharmacological effects. See, for example, U.S. Patent No. 5,861,423 to Caldwell et al. at col. 1, lines 14-44. Various nicotinic compounds also have been reported as being useful for treating a wide variety of conditions and disorders. See, for example, U.S. Patent No. 5,861,423 to Caldwell et al. at col. 1, lines 45-55 and U.S. Patent No. 5,952,339 to Bencherif et al. at col. 1, line 51 through col. 2, line 39. Nicotinic compounds have been recognized as useful in treating a wide variety of central nervous system (CNS) disorders. See, for example, U.S. Patent No. 5,986,100 to Bencherif et al. and U.S. Patent Application Serial No. 09/391,747, filed September 8, 1999.

The distribution and function of nicotinic cholinergic receptors within the body is consistent with the view that nicotinic cholinergic signaling is involved in the regulation of the key neurochemicals in the brain and influences nicotine-sensitive neuronal processes involved in sensory processing and cognition. Major cholinergic systems and subsystems have been described in rodent and primate brains. See, Gotti, *Human Neuronal Nicotinic Receptors. Progress in Neurobiology* **53**: 199-237 (1997). Cholinergic neurons are located in a number of regions throughout the brain, and there are a number of neurotransmitters whose release is modulated by effects upon nicotinic cholinergic receptors.

Certain nicotinic cholinergic receptor subtypes have been recognized as targets for diagnostic imaging. See, Villemagne et al., In: Arneric et al. (Eds.) *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities, 235-250 (1998).* In addition, efforts have been directed toward development of radiotracers that image certain nicotinic receptors within the brain. See, Guan et al., *Journal of Neurochemistry* **74(1)**:237-243 (2000).

There has been reported to be an early and significant depletion of high affinity nicotinic receptors in the brains of Alzheimer's patients. For example, individuals with beta-amyloid plaques show a greater depletion of high affinity nicotinic receptors in the entorhinal cortex than those without such plaques. See, Perry et al., *Annals New York Acad. Sci.* **777**: 388-392 (1996). In addition, selective loss of cholinergic nicotinic receptor subtypes has been reported in post-mortem studies of brains of schizophrenic patients. See, Leonard et al., *Schizophr Bull*: **22(3)**: 431-445 (1996).

There have been efforts to develop non-invasive techniques to probe neuro-receptors *in vivo*. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) of high affinity ligands to map and monitor alterations in receptor densities for a variety of receptor targets having relevance to human diseases has been investigated. For example, studies using ^{11}C-nicotine have demonstrated a decrease in high affinity nicotinic binding sites in post-mortem studies using brain tissues from Alzheimer, Parkinson, and schizophrenic patients. See, Nordberg et al., *Neurosci. Lett.* 72: 115-119 (1986); Kellar etal., *Brain Res.* **436**: 62-68 (1987); Araújo et al., *Neurochemistry* **50**: 1914-1923 (1988); Whitehouse et al., *Arch.*

Neurol. **45**: 722-724 (1988); Whitehouse et al., *Neurol.* **38**: 720-723 (1988); London et al., *Neurochem. Res.* **14**: 745-750 (1989); and Freedman et al., *Biological Psychiatry.* **38**: 22-33 (1995). However, there are recognized limitations of using ^{11}C-nicotine as a ligand for measurement of neuronal nicotinic cholinergic receptors *in vivo.* For example, radiotracer uptake is mostly influenced by regional cerebral blood flow (rCBF), and limitations relating to saturability and short ligand-receptor interaction (a reflection of the binding affinity) have been proposed as the major shortcomings of this ligand. See, Villemagne et al., In*: Alzheimer's Disease; From Molecular Biology to Therapy,* Becker et al. (Eds.), 235-250 (1997). The use of dual tracer using ^{15}O followed by ^{11}C-nicotine has been proposed as a method to circumvent the cerebral blood flow variations. However, the high non-specific binding has further dampened the effort to use a nicotine-based compound as a viable probe.

Some attempts have been made to use a compound known as ^{11}C-ABT-418 as a probe for neuronal nicotinic cholinergic receptors in primates but the results have been disappointing. See. Valette et al., *Nucl. Med. Commun.* **18**: 164-168 (1997). The ^{18}F-labeled analog of a compound known as A-85380 has been investigated for its feasibility as a probe for human neuronal nicotinic cholinergic receptors. See, Valette et al, *J. Nucl. Med.* **40(8)**:1374-1380 (1999). The evaluation of the ^{123}I analog of the compound known as A-85380 as a probe using SPECT has been reported. See, Vaupel et al., *Neuroreport* **13**: 2311-2317 (1998) and Musachio et al., *Nucl. Med. Biol.* **26**: 201-207 (1999). In addition, radiolabeled epibatidine, a nicotine analog, has been reported as having potential use to image nicotinic cholinergic receptors. See, U.S. Patent No. 5,969,144 to London et al. and Villemagne et al., In*: Alzheimer's Disease; From Molecular Biology to Therapy,* Becker et al. (Eds.), 235-250 (1997). Furthermore, ^{76}Br labeled compounds have been proposed as useful diagnostic probes. See, also, Muziere et al., *Life Sci.,* **35**: 1349-1356 (1984); Loc'h et al., *Nucl. Med. Bio.,* **21**: 49-55 (1994); Kassiov, *J. Lab. Cmp. Radiopharm.,* **36(3)**: 259-266 (1995) and Loc'h et al., *Nucl. Med. Bio.,* **23**: 813-819 (1996).

It would be desirable to provide compounds that could act selectively and hence act as probes for the diagnosis of diseases and disorders.

SUMMARY OF THE INVENTION

The present invention, in one aspect, relates to diagnostic compositions. The present invention relates to compounds useful as probes that are useful for determining the relative number and/or function of specific receptors. Of particular interest are nicotinic agonists and antagonists that are selective to certain nicotinic receptor subtypes. In the preferred aspect of the present invention, the compounds are metanicotine-type compounds and azaadamantane-type compounds. The compounds of the present invention most preferably are labeled with a radioactive isotopic moiety such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I or ^{125}I.

In one aspect, the present invention relates to a method for diagnosing disease in a subject, such as a human patient. The method comprises administering to that patient a detectably labelled compound (e.g., a metanicotine-type compound or an azaadamantane-type compound) and detecting the binding of that compound to selected nicotinic receptor subtypes (e.g., alpha 7 and/or alpha 4 beta 2 receptor subtypes).

In another aspect, the present invention relates to a method for monitoring selective nicotinic receptor subtypes of a subject, such as a human patient. The method comprises administering a detectably labeled compound (e.g., a metanicotine-type compound or an azaadamantane-type compound) to that patient and detecting the binding of that compound to selected nicotinic receptor subtypes (e.g., alpha 7 and/or alpha 4 beta 2 receptor subtypes).

In accordance with the present invention, the compounds that have been administered are detected using methods such as position emission topography (PET) and single-photon emission computed tomography (SPECT).

The present invention allows one skilled in the art of the use of diagnosis tools, such as PET and SPECT, to diagnose a wide variety of conditions and disorders, including conditions and disorders associated with dysfunction of the central and autonomic nervous systems. The present invention is useful in the diagnosis of a wide variety of CNS diseases and disorders, including Alzheimer's disease, Parkinson's disease and schizophrenia.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

A compound of the present invention possesses at least one of what is considered to be a radiotracer functionality. Of particular interest are those compounds that include radioactive isotopic moieties such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I, ^{125}I, and the like. Compounds can be radiolabeled at any of a variety of positions. For example, a radionuclide of the halogen series may be used within an alkyl halide or aryl halide moiety or functionality; while a radionuclide such as ^{11}C may be used with an alkyl (e.g., methyl) moiety or functionality.

Compounds of the present invention include those that are nicotinic agonists that possess radiotracer functionalities. Radiolabeled compounds possessing radiotracer functionalities are compounds that possess at least one radioactive isotope as a moiety thereof. Exemplary types of compounds are those of the type set forth in U.S. Patent Nos. 5,212,188 to Caldwell et al., 5,604,231 to Smith et al., 5,616,707 to Crooks et al.; 5,663,356 to Ruecroft et al.; 5,726,316 to Crooks et al.; 5,811,442 to Bencherif et al. and 5,861,423 to Caldwell et al.; and PCT WO 97/40011; 99/65876 and 00/007600; and U.S. Patent Application Serial No. 09/391,747, filed September 8, 1999. The foregoing references are incorporated herein by reference in their entirety for purposes of providing disclosure of representative compounds useful in carrying out the present invention.

Exemplary compounds useful in accordance with the present invention include metanicotine-type compounds; such as:

(3E)-N-[^{11}C]methyl-4-(3-pyridyl)-3-buten-1-amine

(3E)-N-[^{11}C]methyl-4-(5-pyrimidinyl)-3-buten-1-amine

(3E)-N-methyl-4-[5-[^{18}F]fluoro-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[5-[^{123}I]iodo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[5-[^{125}I]iodo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[5-[^{76}Br]bromo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[(6-[^{18}F]fluoro-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[(6-[^{123}I]iodo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[(6-[^{125}I]iodo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-methyl-4-[(6-[^{76}Br]bromo-(3-pyridyl)]-3-buten-1-amine

(3E)-N-[^{11}C]methyl-4-[5-ethoxy-(3-pyridyl)]-3-buten-1-amine

(3E)-N-[^{11}C]methyl-4-[5-methoxy-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-phenoxy-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-methoxymethyl-(3-pyridyl)]-3-buten-1-amine
(3E)-N-methyl-4-[5-[^{11}C]methoxymethyl-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-benzyloxy-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-isobutoxy-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-isopropoxy-(3-pyridyl)]-3-buten-1-amine
(3E)-N-[^{11}C]methyl-4-[5-phenyl-(3-pyridyl)]-3-buten-1-amine
(4E)-N-[^{11}C]methyl-5-[5-isopropoxy-(3-pyridyl)]-4-penten-2-amine
(S)-(4E)-N-[^{11}C]methyl-5-[(5-isopropoxy-(3-pyridyl)]-4-penten-2-amine and
(R)-(4E)-N-[^{11}C]methyl-5-[(5-isopropoxy-(3-pyridyl)]-4-penten-2-amine.

The manner in which the compounds of the present invention can be synthesized can vary. For example, a compound such as N-[^{11}C]methyl-metanicotine ((3E)-N-[^{11}C]methyl-4-(3-pyridyl)-3-buten-1-amine) can be synthesized using the following techniques. The compound can be prepared by a standard N-methylation reaction of the corresponding nor-methyl metanicotine compound ((3E)-4-(3-pyridyl)-3-buten-1-amine) using ^{11}C-labeled methyl iodide. Methods similar to those described by A. G. Horti et al., *J. Med. Chem.* **41**: 4199-4206 (1998) can be used. In general, a large excess of ^{11}C-labeled methyl iodide is used. Typically, the ratio of ^{11}C-labeled methyl iodide to nor-methyl metanicotine is greater than 100:1 and preferably much greater than 1000:1. The resulting N-[^{11}C]methyl-metanicotine can be purified by semi-preparative or preparative HPLC and briefly isolated for reconstitution. The ^{11}C-labeled methyl iodide can be prepared according to the general method described by B. Långström et al. *J. Nucl. Med.* **28(6)**:1037-1040 (1987). Thus, nitrogen gas is irradiated with 10 MeV protons producing ^{11}C-carbon dioxide. The ^{11}C-carbon dioxide is trapped using 4Å molecular sieves, which are subsequently stored in a lead shield. The ^{11}C-carbon dioxide is liberated from the 4Å molecular sieves by heating to ~250°C. The ^{11}C-carbon dioxide is then carried in a stream of nitrogen and trapped in a vessel containing lithium aluminum hydride in tetrahydrofuran. The tetrahydrofuran is removed by heating and a nitrogen flow, and the lithium aluminum hydride complex is then hydrolyzed by treatment with

hydriodic acid, affording ^{11}C-labeled methyl iodide. The ^{11}C-labeled methyl iodide can be transferred by carrier gas to the reaction vessel containing nor-methyl metanicotine. The required nor-methyl metanicotine, (3E)-4-(3-pyridyl)-3-buten-1-amine, can be synthesized according to the procedure described by W. C. Frank et al., *J. Org. Chem.* **43**: 2947-2949 (1978) involving a palladium-catalyzed coupling of 3-bromopyridine and N-3-butenylphthalimide. Removal of the N-phthaloyl protecting group of the resulting Heck reaction product can be accomplished by treatment with hydrazine or methylamine, followed by heating with hydrochloric acid. The required N-3-butenylphthalimide can be synthesized by heating potassium phthalimide and 4-bromo-1-butene in N,N-dimethylformamide. Procedures similar to these are reported in U.S. Patent No. 5,861,423 to Caldwell, et al.

Compounds of the present invention can include metanicotine-type compounds in which the pyridine ring contains a radioisotopic halogen substituent useful for imaging (e.g., ^{18}F, ^{76}Br, ^{123}I or ^{125}I). The manner in which such compounds can be made varies. For instance, (3E)-N-methyl-4-[5-amino-(3-pyridyl)]-3-butene-1-amine, which can be synthesized according to techniques described in U.S. Patent No. 5,597,919 to Dull, et al., can serve as the precursor for the corresponding 5-[^{18}F]fluoro, 5-[^{76}Br]bromo and 5-[$^{123/125}$I]iodo derivatives. The necessary reaction sequence involves protection of the aliphatic amine with an acid-stable protecting group, diazotization of the aromatic amine in the presence of the appropriate radioisotopic halide, and subsequent deprotection of the aliphatic amine. The techniques required for these conversions are similar to those described in U.S. Patent No. 5,510,355 to Bencherif, et al. and by N. Yoneda and T. Fukuhara, *Tetrahedron* **52**: 23-36 (1996).

Compounds of the present invention also include those that are nicotinic antagonists, and preferably those that possess an azaadamantane ring. Antagonists that possess high binding affinity for selective receptor subtypes are desirable probes for radioactive imaging, and can provide a relatively low incidence of pharmacological responses. See, Villemagne et al., In: *Alzheimer's Disease; From Molecular Biology to Therapy,* Becker et al. (Eds.), 235-250 (1997). Exemplary types of compounds are those of the type set forth in U.S. Patent No. 5,986,100 to Bencherif et al., which is

incorporated herein by reference in its entirety for purposes of providing disclosure of representative compounds useful in carrying out the present invention.

Exemplary compounds useful in accordance with the present invention include azadamantane-type compounds; such as:

1-aza-2-[5-[^{123}I]iodo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[5-[^{125}I]iodo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[6-[^{123}I]iodo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[6-[^{125}I]iodo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[5-[^{18}F]fluoro-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[6-[^{18}F]fluoro-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[5-[^{76}Br]bromo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-2-[6-[^{76}Br]bromo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane

1-aza-6-[^{18}F]fluoro -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-5-([^{18}F]fluoromethyl) -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-6-[^{123}I]iodo -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-5-([^{123}I] iodomethyl)-2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-6-([^{125}I]iodo) -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-5-([^{125}I]iodomethyl) -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane

1-aza-6-([^{76}Br]bromo)-2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane and

1-aza-5-([^{76}Br]bromomethyl) -2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane.

Compounds of the present invention can be aryl halides, in which the halogen atom exists as a radioisotope useful for imaging (e.g., ^{18}F, ^{76}Br, ^{123}I, ^{125}I). The methods by which such aryl [^{123}I and ^{125}I]iodides are made can vary. In an approach, described by I. Kampfer et al., *Eur. J. Nuc. Med.* **23**: 157-162 (1996), 1-aza-2-[5-bromo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane serves as the precursor for the corresponding 5-[$^{123/125}$I]iodo derivatives. Thus 1-aza-2-[5-bromo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane can be converted directly into 1-aza-2-[5-[$^{123/125}$I]iodo-(3-pyridyl)] tricyclo[3.3.1.1^{3,7}]decane using commercially available (Amersham) sodium [^{123}I]iodide or sodium [^{125}I]iodide and a mixture of copper(I) chloride, ascorbic acid and stannous sulfate. Alternatively, the 5-bromo precursor can be converted into a 5-trimethylstannyl derivative, which can subsequently be [$^{123/125}$I]iododestannylated to give 1-aza-2-[5-[$^{123/125}$I]iodo-(3-pyridyl)]

tricyclo[3.3.1.1^{3,7}]decane. The conversion of the 5-bromo compound into the 5-trimethylstannyl derivative can be accomplished in two ways, through the action of trimethylstannyl sodium (as described by A. Koren et al., *J. Med Chem.* **41**: 3690-3698 (1998)) or by treatment with hexamethyldistannane and bis(triphenylphosphine)palladium(II) dichloride (as described by H. Saji et al., *Chem. Pharm. Bull.* **45**: 284-290 (1997)). The subsequent [$^{123/125}$I]iododestannylation can be accomplished using commercially available (Amersham) sodium [^{123}I]iodide or sodium [^{125}I]iodide and either hydrogen peroxide (H. Saji et al., *Chem. Pharm. Bull.* **45**: 284-290 (1997)) or chloramine T (J. Musachio, et al., *Life Sciences* **62**: 351-357 (1998)). The unlabeled 5-bromo precursor required for the forgoing methods, 1-aza-2-[5-bromo(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane, is prepared from 3-aminomethyl-5-bromopyridine according to the procedures set forth in PCT WO 99/51602 to M. Bencherif et al. Yet another approach involves conversion of the 5-bromo compound into the corresponding 5-amino compound, 1-aza-2-[5-amino-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane (also described in PCT WO 99/51602 to M. Bencherif et al.) and subsequent transformation into the 5-[$^{123/125}$I]iodo derivatives, by employing diazonium ion techniques familiar to those skilled in the art of organic synthesis. The synthesis of 1-aza-2-[5-iodo-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane is reported in this application as a working example.

Radiolabeled [^{18}F]fluoro compounds of the present invention can also be produced from the 5-amino compound just described. Thus, using synthetic methods similar to those described by N. Yoneda and T. Fukuhara, *Tetrahedron* **52**: 23-36 (1996), 1-aza-2-[5-amino-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane can be diazotized in the presence of H^{18}F and pyridine to give 1-aza-2-[5-[^{18}F]fluoro-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane. The required aqueous H^{18}F solution is generated by 16 MeV proton irradiation of $H_2{}^{18}O$, as described by M. Kilbourn, et al., *Int. J. Appl. Radiat. Isot.* **36**: 327-328 (1985).

The manner varies in which the compounds of the present invention, such as radiolabeled [^{76}Br]bromo compounds, can be synthesized. For example, a compound such as 1-aza-2-[5-[^{76}Br]bromo(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane, can be provided using the following

techniques. In one approach, the radiolabeled [^{76}Br]bromo compound, 1-aza-2-[5-[^{76}Br]bromo(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane can be prepared by bromodestannylation of the corresponding 5-trimethylstannyl precursor using [^{76}Br]bromide and an oxidant such as hydrogen peroxide or chloramine-T. Methods similar to those described by J. Koziorowski et al., *J. Radioanal. Nucl. Chem.*, **219(1)**: 127-128 (1997) and C. Loc'h et al., *Nucl. Med. Biol.* **23**: 813-819 (1996) can be used. The required trimethylstannyl compound, 1-aza-2-[5-trimethylstannyl(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane can be prepared by treatment of the previously described 5-bromo compound, 1-aza-2-[5-bromo(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane with hexamethyldistannane and bis(triphenylphosphine)palladium(II) dichloride according to methods similar to those described by J. Koziorowski et al., *J. Radioanal. Nucl. Chem.* **219(1)**: 127-128 (1997) and H. Saji et al., *Chem. Pharm. Bull.* **45(2)**: 284-290 (1997). The required [^{76}Br]bromide can be prepared according to methodology described by J. Koziorowski et al., *J. Radioanal. Nucl. Chem.* **219(1)**: 127-128 (1997). Alternatively, [^{76}Br]bromide can be produced from copper(I) selenide by irradiation with a low-energy cyclotron according to the methodology described by V. Tolmachev et al., *Appl. Radiat. Isot.* **49(12)**: 1537-1540 (1998). This method produces primarily [^{76}Br]bromide. The production of [^{76}Br]bromide can also be accomplished from natural arsenic using the method described by M. Kassiou et al., *J. Lab. Comp. Radiopharm.* **XXXVI**: 259-266 (1995). Thus, natural arsenic can be irradiated with a beam of 30 MeV [^{3}He] ions. After cooling 15 hours to allow [^{75}Br]species to decay, the target can be dissolved in sulfuric acid and oxidized with chromic acid. The resulting [^{76}Br]bromine can be carried via nitrogen and trapped in ammonia as ammonium [^{76}Br]bromide. The resulting solution can be evaporated to dryness and later reconstituted, typically as an aqueous solution containing ammonium [^{76}Br]bromide.

Another approach to radiolabeled [^{76}Br]bromo compounds of the present invention can be accomplished by proceeding from the iodinated precursor, 1-aza-2-[5-iodo(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane, the preparation of which has been previously described. Bromodeiodination methods similar to those described by C. Loc'h et al., *Nucl. Med. Biol.* **21**: 49-55 (1994) can be used. Thus, 1-aza-2-[5-iodo(3-pyrridyl)]tricyclo[3.3.1.1^{3,7}]decane can be

converted to 1-aza-2-[5-[^{76}Br]bromo(3-pyrridyl)]tricyclo[3.3.1.1^{3,7}]decane by heating at 165°C with ammonium [^{76}Br]bromide and copper sulfate pentahydrate in a copper(I) assisted nucleophilic aromatic substitution reaction. Ammonium [^{76}Br]bromide can be obtained as previously described.

Compounds of the present invention include radiolabeled aryl halides in which the halide moiety resides in a ring position other than the 5 position (i.e., the 2, 4, and 6 positions). The methods by which these compounds can be made are similar to those previously described, in which an amino precursor is subjected to diazotization in the presence of appropriate radioisotopic halide. For instance, 1-aza-2-[6-amino-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane can be transformed, by methods similar to those described by N. Yoneda and T. Fukuhara, *Tetrahedron* **52**: 23-36 (1996), into 1-aza-2-[6-[^{18}F]fluoro-(3-pyridyl)]tricyclo[3.3.1.1^{3,7}]decane. Numerous analogous procedures for the conversion of the aryl amines into the corresponding aryl bromides and iodides are reported in the synthetic literature (see *The Chemistry of Diazonium and Diazo Groups*, Part 1, pp 288-290, J. Wiley and Sons, 1978). Many of these techniques are amenable to the use of radioisotopic bromide and iodide starting materials. The required 6-amino compound can be generated from 1-aza-2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane by the Chichibabin reaction using procedures similar to those reported by B. Latli, et al., *J. Med Chem.* **42**: 2227-2234 (1999). Typically the Chichibabin reaction gives mixtures of isomeric (2 and 4 positions relative to the ring nitrogen) pyridyl amines (*Chemistry of Heterocyclic Compounds*, Volume 14, part 3, pp. 3-5, Interscience Publishers, 1962). These positional isomers can typically be separated from one another and carried on separately through the diazotization process, as described in B. Latli, et al., *J. Med Chem.* **42**: 2227-2234 (1999). Such a sequence of synthetic steps could be used to generate 1-aza-2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decanes with radioisotopic halogen substitution in the 2 and 4 positions of the pyridine ring. The synthesis of the required 1-aza-2-(3-pyridyl)tricyclo[3.3.1.1^{3,7}]decane (reactant for the Chichibabin reaction) is described in PCT WO 99/51602 to M. Bencherif et al.

Compounds of the present invention include radiolabeled alkyl halides derived from the azaadamantane skeleton. The manner in which these compounds can be made varies. For instance, the ketone functionality of 5-aza-1-(hydroxymethyl)-6-(3-pyridyl)tricyclo[3.3.1.$1^{3,7}$]decan-2-one (the synthesis of which is described in PCT WO 99/51602 to M. Bencherif et al.) can be reduced, through the intermediacy of the *p*-toluenesulfonylhydrazone, to the alkane. Procedures which are benign to alcohol functionality, such as those reported by L. Anzalone and J. Hirsch, *J. Org. Chem.* **50**: 2607-2613 (1985) and by L. Caglioti, *Organic Syntheses* **Coll. Vol. 6**: 62-63 (1988), can be used for this purpose. Subsequent conversion of the remaining alcohol functionality to the corresponding methanesulfonate, *p*-toluenesulfonate or trifluoromethanesulfonate ester can be accomplished using techniques familiar to those skilled in the art of organic synthesis. These esters can be transformed, by treatment with the appropriate radioisotopic halide, into radioisotopic alkyl halides. Thus, 1-([^{18}F]fluoromethyl)-5-aza-6-(3-pyridyl)tricyclo[3.3.1.$1^{3,7}$]decane can be made by nucleophilic displacement of the corresponding trifluoromethanesulfonate ester with K^{18}F/Kryptofix, similar to the procedures described by J. Grierson and A. Shields, *J. Nuc. Med.* **39**: 22P (1998) and K. Bergmann, et al., *Nucl. Med. Biol.* **21**: 25-39 (1994). The corresponding [^{76}Br]bromo and [$^{123/125}$I]iodo compounds can be made by similar displacement reactions, familiar to those skilled in the art of organic synthesis, using the appropriate radioisotopic halide nucleophiles.

In another application of this technology, a ketone functionality can be reduced to an alcohol functionality, which subsequently can be converted into an alkyl halide. For instance, 5-aza-6-(3-pyridyl)tricyclo[3.3.1.$1^{3,7}$]decan-2-one (the synthesis of which is described in PCT WO 99/51602 to M. Bencherif et al.) can be reduced with sodium borohydride in methanol, as described for the reduction of camphor in *Introduction to Organic Laboratory Techniques*, Second Edition, p 156, Saunders College Publishing Co., to afford 5-aza-6-(3-pyridyl)tricyclo[3.3.1.$1^{3,7}$]decan-2-ol as a mixture of chromatographically inseparable diastereomers (see PCT WO 99/51602 to M. Bencherif et al.). This mixture of alcohols can be transformed into a corresponding mixture of diastereotopic halides using techniques described above (i.e., conversion into the methanesulfonate, *p*-toluenesulfonate or trifluoromethanesulfonate ester

and subsequent displacement with halide). Through the use of radioisotopic halide ([^{18}F]fluoride, [^{76}Br]bromide or [$^{123/125}$I]iodide), compounds of the present invention can be generated.

The present invention relates to radiotracer compositions incorporating relevant amounts of a compound of the present invention. Appropriately radiolabeled compounds are administered to a subject (e.g., a human subject), and the presence of that compound within the subject is imaged and quantified by appropriate techniques in order to indicate the presence, quantity and functionality of selected nicotinic cholinergic receptor subtypes. For administration to humans, compounds that include radioactive isotopic moieties such as ^{11}C, ^{18}F, ^{76}Br or ^{123}I are particularly preferred. Compounds of the present invention also can be administered to animals, such as mice, rats, dogs and monkeys. For administration to animals, compounds that include radioactive isotopic moieties such as ^{11}C, ^{18}F, ^{76}Br, ^{123}I and ^{125}I are particularly preferred. The present invention also relates to a method for quantitating the relative number of selected nicotinic cholinergic receptor subtypes. As such, it is possible to diagnose certain conditions, disorders or diseases.

The compounds can be administered in a free base form or in the form of salts. Representative salts are organic or inorganic acid addition salts of the type set forth in U.S. Patent Nos. 5,663,356 to Ruecroft et al.; 5,861,423 to Caldwell et al. and 5,986,100 to Bencherif et al., which are incorporated herein by reference in their entirety.

The compounds are administered using known techniques. See, for example, U.S. Patent No. 5,969,144 to London et al. The compounds can be administered in formulation compositions that incorporate other ingredients, such as those types of ingredients that are useful in formulating a diagnostic composition. Compounds useful in accordance with carrying out the present invention most preferably are employed in forms of high purity. See, U.S. Patent No. 5,853,696 to Elmalch et al. The compounds are administered in appropriate doses. Determination of dose is carried out in a manner known to one skilled in the art of radiolabel imaging. See, for example, U.S. Patent No. 5,969,144 to London et al.

The compounds are labeled with radionuclides useful in PET (e.g., ^{11}C, ^{18}F or ^{76}Br) and SPECT (e.g., ^{123}I) imaging, with half-lives of about 20.4 minutes for ^{11}C, about 109 minutes for ^{18}F, about 13 hours for ^{123}I, and about 16 hours for ^{76}Br. A high specific activity is desired to visualize the selected receptor subtypes at non-saturating concentrations. The administered doses typically are below the toxic range and provide high contrast images. The compounds are expected to be capable of administration in non-toxic levels.

SPECT and PET imaging may be carried out using any appropriate technique and apparatus. See Villemagne et al., In: Arneric et al. (Eds.) *Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities, 235-250 (1998) and* U.S. Patent No. 5,853,696 to Elmalch et al. for a disclosure of representative imaging techniques.

The compounds that are employed in accordance with the present invention most preferably are selective to certain nicotinic cholinergic receptor subtypes. Preferred compounds bind with high affinity to selective nicotinic cholinergic receptor subtypes (e.g., alpha 4 beta 2 and alpha 7) and exhibit negligible non-specific binding to other nicotinic cholinergic receptor subtypes (e.g., those receptor subtypes associated with muscle and ganglia). As such, compounds of the present invention can be used as agents for noninvasive imaging of nicotinic cholinergic receptor subtypes within the body of a subject, particularly within the brain for diagnosis associated with a variety of CNS diseases and disorders. Representative diseases and disorders that can be evaluated in accordance with the present invention include those that are set forth in U.S. Patent No. 5,952,339 to Bencherif et al. and U.S. Patent Application Serial No. 09/391,747, filed September 8, 1999.

The following example is provided to further illustrate the present invention, but should not be construed as limiting the scope thereof. Unless otherwise noted, all parts and percentages are by weight.

Example 1

Concentrated hydrochloric acid (0.25 mL, 3.0 mmol) was added drop-wise to a ice bath cooled suspension of 1-aza-2-[5-amino-(3-pyridyl)]tricyclo[3.3.1.$1^{3,7}$]decane (the preparation of which is described in PCT WO 99/51602 to M. Bencherif et al.) (110 mg, 0.480 mmol) in water

(0.75 mL). To the resulting light yellow solution, ice bath cooled, was added a solution of sodium nitrite (40 mg, 0.58 mmol) in water (0.25 mL). After stirring for 5 min, the now dark yellow solution (still in the ice bath) was treated dropwise with a solution of potassium iodide (90 mg, 0.54 mmol) in water (0.25 mL). The mixture immediately turned brown, evolved gas and precipitated a brown solid. The mixture was warmed to 10°C over a period a 1 h and treated sequentially with sodium thiosulfate (45 mg, 0.28 mmol) in water (0.25 mL) and enough 10% aqueous sodium hydroxide at make the mixture strongly basic (>pH 10). The mixture was then extracted with chloroform (3 x 10 mL), and the chloroform extracts were dried (Na_2SO_4) and concentrated, leaving 143 mg of dark brown gum. Column chromatography on 10g of Merck silica gel 60 (70-230 mesh) with 0-4% methanol, 2% triethylamine in benzene gave 81 mg of waxy white solid. The solid was dissolved in ethanol (4 mL) and cooled in ice as concentrated hydrochloric acid (0.25 mL) was slowly added. The volatiles were evaporated, first by rotary evaporation, then by high vacuum treatment. The residue was dissolved in hot ethanol (6 mL), diluted with isopropanol (2 mL), and cooled in the freezer (-4°C) overnight. The suspension was suction filtered, and the resulting white powder was vacuum dried (40°C, 16 h), providing 76 mg (38% yield) of 1-aza-2-[5-iodo-(3-pyridyl)]tricyclo[3.3.1.$1^{3,7}$]decane dihydrochloride (mp 226-230°C, with sublimation). The compound exhibits a Ki, as determined using the techniques set forth in U.S. Patent No. 5,986,100, of 14 nM.

What Is Claimed Is:

1. A method for diagnosing disease in a subject, the method comprising administering to the subject a detectably labeled compound and detecting the binding of that compound to nicotinic receptor subtypes.

2. A method of monitoring selective nicotinic receptor subtype of a subject, the method comprising administering a detectably labeled compound to the subject.

3. The method of Claim 1 or 2 whereby the compound is detected using position emission topography.

4. The method of Claim 1 or 2 whereby the compound is detected using single-photon emission computed tomography.

5. The method of Claim 1 or 2 whereby the subject is a human patient.

6. The method of Claim 1or 2 whereby the subject is an animal.

7. The method of Claim 5 whereby the detectably labeled compound comprises a moiety selected from the group consisting of ^{11}C, ^{18}F, ^{76}Br, and ^{123}I.

8. The method of Claim 6 whereby the detectably labeled compound comprises a moiety selected from the group consisting of ^{11}C, ^{18}F, ^{76}Br, ^{123}I and ^{125}I.

9. The method of Claim 1 or 2 whereby the compound is a metanicotine-type compound.

10. The method of Claim 1 or 2 whereby the compound is an azaadamantane-type compound.

11. The method of Claim 1 or 2 whereby the compound is selective to an alpha 4 beta 2 receptor subtype.

12. The method of Claim 1 or 2 whereby the compound is selective to an alpha 7 receptor subtype.

13. The method of Claim 1 or 2, whereby the disease is selected from the group consisting of Alzheimer's disease, Parkinson's disease and schizophrenia.

14. The method of Claim 9, whereby the the detectably labeled compound comprises a moiety selected from the group consisting of ^{11}C, ^{18}F, ^{76}Br, ^{123}I and ^{125}I.

15. The method of Claim 10, whereby the detectably labeled compound comprises a moiety selected from the group consisting of ^{11}C, ^{18}F, ^{76}Br, ^{123}I and ^{125}I.

16. The method of Claim 15, whereby the moiety is in a 5 position on its ring.